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Increased VLDL in nephrotic patients results from a decreased catabolism while increased LDL results from increased synthesis

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Increased VLDL in nephrotic patients results from a decreased catabolism while increased LDL results from increased synthesis. Increased very low density lipoprotein (VLDL) in nephrotic patients results from a decreased catabolism while increased low density lipoprotein (LDL) results from increased synthesis. Hyperlipidemia is a hallmark of nephrotic syndrome that has been associated with increased risk for ischemic heart disease as well as a loss of renal function in these patients. The hyperlipidemia usually is characterized by increased cholesterol levels, although hypertriglyceridemia may be present as well. The factors that determine the phenotype of nephrotic dyslipidemia are not understood, nor has the primary stimulus for nephrotic hyperlipidemia been identified. One hypothesis is that nephrotic hyperlipidemia is the result of a coordinate increase in synthesis of proteins by the liver. To address these issues we simultaneously measured the *in vivo* rate of VLDL apolipoprotein B100 (apo B100) secretion, LDL apo B100 synthesis and albumin synthesis in patients with a nephrotic syndrome ($N = 8$) and compared them with a control group ($N = 7$) using a primed/continuous infusion of the stable isotope L-[1-^{13}C] valine for six hours. Kinetic data were analyzed by multicompartmental analysis. Patients studied had combined hyperlipidemia as reflected by an significant increase in both VLDL and LDL apo B100 pool sizes. In contrast, the albumin pool size was significantly decreased. VLDL apo B100 levels were primarily increased as a consequence of a decrease in fractional catabolic rate (FCR) rather than from an increase in the absolute synthesis rate (ASR). Both VLDL apo B100 and triglycerides were inversely related to the fractional catabolism (FCR) of VLDL apo B100 ($r^2 = 0.708$; $P = 0.0088$) while neither had any relationship to the ASR of VLDL apo B100. In contrast to VLDL, increased LDL apo B100 was not a consequence of decreased catabolism. The LDL apo B100 ASR was significantly increased ($P = 0.001$) in the nephrotic patients compared to controls. Low density lipoprotein apo B100 ASR was greater than that of VLDL apo B100 in some patients, suggesting that LDL in these patients was not only derived from VLDL delipidation, but also by an alternative secretory pathway. There was no clear relationship between the ASR of VLDL apo B100 and the ASR of albumin within the current study population. Our data indicate that increased VLDL in nephrotic patients results from a decreased catabolism, while increased LDL results from increased synthesis.

Key words: stable isotopes, nephrotic syndrome, apo B100, albumin, lipoproteins and nephrotic syndrome.

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Hyperlipidemia is one hallmark of the nephrotic syndrome (NS) [1]. Hyperlipidemia in the nephrotic syndrome has been proposed to result from increased synthesis and decreased catabolism of lipoproteins [2–5], although the relative contribution of each has not been quantitated. The mechanism of hyperlipidemia is of clinical significance, since hyperlipidemia has been implicated in increased cardiovascular risk and in progression of renal injury in nephrotic patients [6–8]. Although an increase in total plasma cholesterol is the most common abnormality, plasma triglyceride (TG) levels are also increased in patients with more severe proteinuria [4, 9, 10], but this increase is not invariably present. It is not known why some patients with the nephrotic syndrome develop increased TG levels while others do not.

While some investigators have reported an increased rate of very low density lipoprotein/low density lipoprotein (VLDL/LDL) secretion, others have not been able to confirm this finding. This could be due to differences in methodology or to differences in the patient population studied. Direct measurement of VLDL catabolism using ^{125}I labeled lipoproteins is complicated by the fact that VLDL contains several apolipoproteins and more than one may be labeled. Of greater concern is that most studies employing iodination of proteins use oxidizing reagents that might modify lipids and may lead to different processing (such as scavenger receptor) so that they are processed differently than native lipoproteins. We avoided these potential artifacts by directly measuring synthesis and secretion of apolipoprotein B100 (apo B100) in nephrotic patients using stable isotopes. Although stable isotope measurements require extensive protein isolation procedures, complex multicompartmental modeling and specific equipment, no radiation is involved and this method has been proven to be a feasible approach to measure apo B metabolism [5, 11, 12].

Synthesis of a group of proteins, including albumin, is increased proportionally in the nephrotic syndrome in experimental animals [13–15] and in humans [16–18], leading to the hypothesis by Marsh and Sparks [19] that hyperlipidemia might be a result of a coordinated increase in synthesis of albumin and other proteins by the liver, including lipoproteins. In addition, Davis et al [20] suggested that low albumin states might exert an effect on hepatic

synthesis of apo B100 by less direct means, specifically by increasing the availability of free fatty acids.

In the present study, synthesis of apo B100 in VLDL and LDL was therefore measured directly and simultaneously with that of albumin synthesis in patients with nephrotic syndrome with a wide range of proteinuria, albumin and hyperlipidemia, using endogenous labeling with infused ^{13}C valine as a precursor.

METHODS

Subjects

Eight patients (6 males, 2 females) were recruited for the study from the renal division of the University Hospital Utrecht. The mean age was 48 ± 4 years (median 51; range 31 to 63 years). All had a stable nephrotic syndrome for at least three months duration. Six of them had membranous glomerulonephritis, one had steroid-resistant minimal change disease and one had focal glomerular sclerosis. Patients were prescribed a diet containing 0.8 g protein/kg body wt/day and 100 mmol sodium/day for at least two weeks before starting the infusion study. Besides diuretics, none of them received medication, or medication was stopped at least two weeks before the infusion study. Control studies were done in seven healthy subjects (3 males, 4 females) who were on a similar diet as the nephrotic syndrome patients. The mean age of the control group was 33 ± 2 years (median 33; range 25 to 40 years). One day before the infusion study, the subjects collected a 24-hour urine sample that was analyzed for urea, creatinine, protein and albumin. All patients and volunteers agreed to participate after signing an informed consent form, in accordance with the Helsinki Declaration of Human Rights. This study was approved by the Institutional Ethical Committee for studies in humans.

MATERIALS

L-[1-^{13}C] valine (isotope mole fraction > 0.99; MassTrace, Woburn, MA, USA) was dissolved in sterile 0.9% saline and sterilized through a 0.22 μm filter. All chemicals were obtained from Riedel de Haën (Seelze, Germany) unless indicated otherwise. Density solutions were made with KBr in 0.9% NaCl, NaN_3 (0.01%) and 1 mmol/liter EDTA.

Methods

Infusion protocol. The infusion protocol has been described in detail previously [18]. In short, samples were taken to measure the Evans blue distribution volume, an index for plasma volume [21]. At the baseline (time, t = 0), a priming dose of 15 μmol/kg L-[1-^{13}C] valine was administered intravenously over two minutes, followed by a continuous infusion of 15 μmol/kg/hr L-[1-^{13}C] valine during six hours. Blood samples (5 ml) were collected into heparin-containing tubes and into EDTA containing tubes (10 ml). Samples were taken from the contralateral arm at t = 0, 15, 30, 60, 120, 180, 240, 270, 300, 330 and 360 minutes. Samples were kept on ice (maximum 1 hr) until plasma was separated by centrifugation (20 min, 3000 rpm, 4°C). Plasma samples for isolation of albumin and lipoproteins were immediately stored at −80°C or used directly.

Isolation of free amino acids, albumin and apo B100 from VLDL and LDL. The isolation of free amino acids from plasma and the isolation of albumin from heparin plasma, which was based on differential solubility in absolute ethanol from TCA-precipitated proteins, was performed as described in detail previously [18]. Lipoproteins were isolated from plasma by sequential ultracentrifugation. For the VLDL isolation, 3.0 ml EDTA plasma was over layered by 2.8 ml of a density solution 1.006 kg/liter and ultracentrifugated for 20 hours at 40,000 rpm in a 50.3 ti rotor (Beckman Instruments, Inc., Palo Alto, CA, USA). After tube slicing, the supernatant was washed by again adding 2.8 ml of the density solution 1.006 kg/liter to 3 ml supernatant. The VLDL fraction was obtained after centrifugation and tube slicing again.

Low density lipoprotein was isolated as described elsewhere [22]. In short, 2.5 ml infranatant of the first run was adjusted with KBr to a density of 1.24 kg/liter and overlayered by 5 ml of a density solution 1.12 kg/liter, 2.0 ml of a density solution 1.06 kg/liter and 2.5 ml of a density solution 1.006 kg/liter followed by ultracentrifugation using a swinging out bucket rotor (SW-40; Beckman). Density bands were aspirated in 0.5 ml fractions. Fraction 13 to 21 contained LDL that was free from intermediate density lipoprotein (IDL) and high density lipoprotein (HDL). Fractions with Lp(a) contents < 28 mg/liter (detection limit) were taken. Control experiments were performed and showed that enrichment in this fraction is a good representation of the total LDL pool. In two patients who had a detectable Lp(a) concentration in this fraction, the sample was pre-treated with lysine-sepharose to remove the Lp(a) (< 28 mg/liter).

Apolipoprotein B100 was isolated from the VLDL and LDL fractions by precipitation with isopropanol as described by Egusa et al [23]. The precipitate was delipidated with ethanol/ether (3:1) and thereafter with ether. At each step of the extraction, the protein-solvent mixture was incubated at −20°C overnight and was centrifugated at 2000 rpm for 30 minutes. The solvent was removed by aspiration. After evaporation the remaining ether fraction, apo B100 was hydrolyzed with 6 N HCl for 24 hours at 110°C [24]. The hydrolysates were supplied to cation-exchange resin as described previously [18].

Quantification. For quantification of lipids and lipoproteins, plasma samples were subjected to a single ultracentrifugation step according to Redgrave, Roberts and West [25]. Plasma, VLDL, IDL and LDL fractions were assayed for total cholesterol and triglyceride on a Synchron CX4 (Beckman). The amount of HDL was measured by precipitating a small aliquot of the bottom fraction using dextran sulphate-Mg^{2+} [26]. Plasma was assayed for apo A1 and for apo B100 using a routine nephelometric assay (Behringwerke AG, Marburg, Germany). For the measurement of apo B100 in the VLDL and IDL fractions, a nephelometric assay was used in which the detection limit of the apo B100 assay was 12 mg/liter.

Determination of ^{13}C-valine enrichment. Derivatization of the isolated amino acids and of the isolated apo B100 was done according to the method of Hušek [27]. The N(O,S)-methoxycarbonyl methyl ester derivatives of plasma free amino acids and of apo B100 from VLDL were analyzed by gas chromatography/mass spectrometry, and the derivatives of apo B100 LDL and of albumin were analyzed by gas chromatography combustion isotope ratio mass spectrometry as described previously [18].

Calculation/statistics. The apo B100 valine tracer/tracee ratio in VLDL and LDL were analyzed by mathematical compartmental modeling (Fig. 1) using SAAM II software [28]. The model consists of an plasma amino acid pool, a delay compartment that accounts for the time delay in appearance of VLDL apo B 100

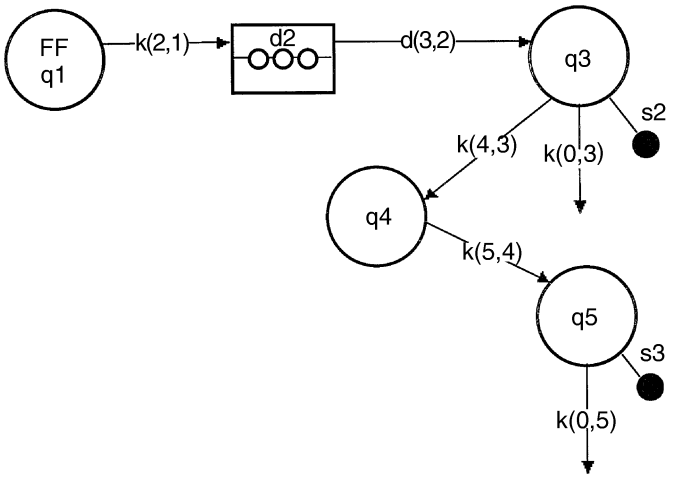

Fig. 1. Multicompartmental model for apolipoprotein (apo) B100 metabolism. The q1 represents the plasma valine pool (FF = forcing function), d2 is a delay compartment, q3 is the very low density lipoprotein (VLDL) compartment, q4 is the intermediate density lipoprotein (IDL) compartment, and q5 is the low density lipoprotein (LDL) compartment; s2 and s3 represent sampling points for determination of the tracer/tracee ratio of the ^{13}C valine in VLDL apo B100 and in LDL apo B100 compartment respectively. The k-value (2,1) represents the fractional synthesis rate (FSR) of VLDL apo B100, and k-value (0,5) the fractional catabolic rate (FCR) for LDL apo B100.

into plasma, and a series of delipidation compartments representing VLDL, IDL and LDL pools. In this model k-values represent the fractional rate at which ^{13}C valine appeared into various apo B100 lipoprotein pools. Simulations were done using the plasma valine tracer/tracee ratio as a forcing function to the model, which allowed an adjustment of the various k-value for an optimal fit to the VLDL apo B100 and LDL data simultaneously. The k-value (2,1) represents the fractional rate of synthesis (FSR) of apo B VLDL. The k-value (0,5) represents the fractional rate of catabolism (FCR) of LDL.

Since the measurements were done while apo B100 metabolism was in steady state, the fractional clearance rate was equal to the fractional production rate (FCR = FSR). The tracer/tracee data of albumin were analyzed by fitting the data to a multicompartmental model consisting of a plasma amino acid pool a delay element and an albumin pool [29].

The absolute synthesis rate (ASR), which is the amount of protein synthesized per day, were calculated by multiplying pool sizes by the corresponding FSR. Pool sizes were calculated by multiplying the plasma concentrations of albumin, VLDL apo B100 and LDL apo B100 with the plasma volume measured by Evans blue. To adjust for different body wts, these quantities are expressed per kg body wt.

Statistics were performed using *t*-test. If the normality test or equal variance test failed a Mann-Whitney Rank sum test was used. All data are presented as means ± SEM

Correlations were performed by linear regression analysis.

RESULTS

Clinical and biochemical parameters

As expected, plasma albumin concentration and plasma colloid oncotic pressure (COP) were significant decreased compared to the control group (Table 1). Creatinine clearance was also decreased significantly. Urine urea excretion, as index of protein intake, was not significantly different from that in the control subjects. Total triglyceride (TG), VLDL-TG, IDL-TG, total cholesterol (chol), VLDL-chol, IDL-chol and LDL-chol were significantly elevated in the patients compared to controls (Table 2). The plasma apo B 100 pool was significantly ($P < 0.001$) increased in the nephrotic patients (2.39 ± 0.29 g/liter) compared to the control group (1.03 ± 0.09 g/liter), while HDL-chol (Table 2) and its apolipoprotein A1 (1.45 ± 0.15 g/liter vs. 1.26 ± 0.08 g/liter) were not significantly different between nephrotic patients and controls. Cholesterol levels were increased above the normal range in all patients and ranged from 7.3 to 15.9 mmol/liter. In contrast, TG levels ranged from 1.0 to 7.7 mmol/liter. Very low density lipoprotein apo B100 pool size was increased above the maximum value found in control subjects in six nephrotic patients, but was within the normal range in two (No. 7 and 8). Pool sizes of LDL apo B100 were significantly increased while the pool sizes of albumin were significantly decreased (Table 3) compared to normal subjects.

Very low density lipoprotein apolipoprotein B100

Enrichment in plasma free valine reached a plateau after 60 minutes and remained stable during the study, for both patients and controls (data not shown). The mean coefficient of variation of plateau was 1.5%. The fraction rate of appearance of ^{13}C valine in VLDL apo B100 (and thereby the FSR) tended to be lower in patients than controls (data not shown). Since in steady state FSR is equal to fractional catabolic rate (FCR), the FCR of VLDL apo B100 tended to be lower in the entire patient group, although this value did not reach significance (Table 3 and Fig. 2A). Within the group of six patients with elevated levels of VLDL apo B100, the FCR was significantly decreased (13.6 ± 3.6%/hr in nephrotic vs. 26.5 ± 3.2%/hr in controls, $P = 0.02$). Since the VLDL apo B100 pool size was increased more than fivefold in nephrotic patients, the absolute rate of ^{13}C valine incorporation was greater in the nephrotic group compared to controls (Table 3 and Fig. 2B). In four nephrotic patients (No. 2, 3, 5 and 6), the calculated ASR of VLDL apo B100 was above the highest value found in the control group, while the remaining patients (No. 1, 4, 7 and 8) had a calculated ASR of VLDL apo B100 similar to those found in the control group (Fig. 4B). In the patient group, plasma VLDL apo B100 concentration correlated inversely with VLDL apo B 100 FCR (Fig. 3A; $r^2 = 0.708$; $P = 0.0088$) and bore no relationship to VLDL apo B 100 ASR (Fig. 3B; $r^2 = 0.245$; $P = 0.21$), suggesting that the expanded VLDL apo B 100 pool size was a consequence of a decrease in FCR, rather than an increase in ASR. Furthermore, the FCR of VLDL apo B100 correlated with plasma VLDL-chol ($r^2 = 0.716$; $P = 0.0081$), plasma VLDL-TG ($r^2 = 0.784$; $P = 0.019$) and total plasma triglyceride ($r^2 = 0.776$; $P = 0.0038$). Those nephrotic patients whose triglycerides were increased all had a decreased FCR of VLDL apo B100, while those whose triglycerides were within the normal range all had a FCR of VLDL apo B100 that was not reduced. The VLDL apo B100 concentration did not correlate with either albumin concentration or urinary albumin secretion ($r^2 = 0.060$; $P = 0.559$ and $r^2 = 0.071$; $P = 0.522$, respectively).

Low density lipoprotein apolipoprotein B100

Low density lipoprotein apolipoprotein B100 concentration and pool size was increased more than twofold in the patient group

Table 1. Clinical characteristics of the patients and control subjects

	Renal disease	Age *years*	Gender	Weight *kg*	Body mass index *kg/m²*	Plasma albumin *g/liter*	COP *mm Hg*	Creatinine clearance *ml/min*	Urinary urea *mmol/day*	Proteinuria *g/day*
Patient no.										
1	membr	49	m	82.1	26.5	21.5	10.7	111	261	12.9
2	membr	52	m	86.2	25.2	21.5	11.7	82	146	6.9
3	membr	31	m	73.6	21.7	23.9	11.8	84	254	6.1
4	membr	56	m	92.0	31.8	24.0	11.7	86	332	16.5
5	membr	41	m	85.2	25.2	19.9	9.5	67	388	11.1
6	membr	55	f	96.7	33.9	24.4	12.3	109	421	23.2
7	focal glom	63	f	70.8	25.1	20.6	10.6	62	218	7.4
8	min ch	40	m	68.6	21.4	26.1	15.1	124	382	2.9
Mean ± SEM		48[a]	6m/2f	81.9	26.3	22.7[b]	11.7[b]	91[a]	300	10.9[b]
		4		3.6	1.6	0.8	0.6	8	34	2.3
Controls										
(N = 7)		33	3m/4f	77.5	24.3	36.9	24.6	137	348	<0.1
Mean ± SEM		2		5.5	1.5	0.8	0.5	9	40	0

Abbreviations are: membr, membranous glomerulonephritis; focal glom, focal glomerulosclerosis; min ch, minimal change disease; COP, colloid oncolic pressure.
[a] $P < 0.01$, [b] $P < 0.0001$

Table 2. Levels of plasma lipids, lipoproteins and apolipoproteins A1 and B100 of both groups

	TG	VLDL-TG	IDL-TG	Chol	VLDL-chol	IDL-chol	LDL-chol	HDL-chol	Apo A1	Apo B100
	mmol/L								*g/liter*	
Patient No.										
1	7.7	4.3	0.4	13.9	2.1	2.0	9.0	0.9	1.70	2.80
2	6.0	3.6	0.5	13.1	3.1	2.3	7.1	0.7	1.12	2.29
3	4.0	2.4	0.4	15.6	2.0	3.2	9.7	0.7	1.17	3.25
4	5.6	2.8	0.3	7.3	1.4	0.7	4.7	0.5	0.92	1.57
5	2.4	0.9	0.5	8.4	0.9	2.5	4.0	1.0	1.18	1.64
6	3.7	1.2	0.6	15.9	1.9	4.2	8.4	1.4	2.18	3.36
7	2.4	0.3	0.4	15.3	0.4	4.0	10.1	0.9	1.63	2.94
8	1.0	0.04	0.1	7.3	0.05	1.6	4.2	1.4	1.67	1.30
Mean ± SEM	4.1[b]	1.9[a]	0.4[b]	12.1[c]	1.5[a]	2.6[b]	7.1[c]	0.9	1.45	2.39[c]
	0.8	0.6	0.1	1.3	0.4	0.4	0.9	0.1	0.15	0.29
Controls										
(N = 7)	1.3	0.3	0.14	5.2	0.3	0.9	3.0	1.1	1.26	1.03
Mean ± SEM	0.1	0.1	0.02	0.3	0.1	0.1	0.2	0.1	0.08	0.09

Abbreviations are in the **Appendix**.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ significantly different from control group

compared to controls. In contrast to VLDL apo B100, the ASR of LDL apo B100 was significantly ($P < 0.001$) increased from a mean of 6.0 ± 0.8 mg/kg/day in the control subjects to 28 ± 7 mg/kg/day in 7 nephrotic patients (Fig. 4B). The FCR of LDL could not be fitted by the model that we used in one patient (No. 4). This finding caused us to consider the possibility of a different pathway for synthesis of LDL from the usually accepted VLDL-IDL-LDL route in this patient. The increase in LDL apo B100 synthesis was responsible for the increase in LDL apo B100 concentration and pool size, since the FCR of LDL apo B100 actually tended to be higher in patients than controls, although this difference did not achieve significance, again due to the wide range in the individual values (Table 3 and Fig. 4A). The absolute rate of LDL apo B100 synthesis was greater than the rate of synthesis of VLDL apo B100 in three of the nephrotic patients (Table 3), suggesting that the relationship between VLDL and LDL synthesis is not a direct precursor product relationship, but instead that at least a component of the LDL pool arises either directly or by a pathway that does not include VLDL. In the patient group there was no relationship between the ASR of albumin and that of apo B100 in LDL or between plasma albumin concentration and the ASR of LDL apo B100 ($r^2 = 0.017$; $P = 0.779$ and $r^2 = 0.026$; $P = 0.728$, respectively).

Albumin

In contrast to VLDL apo B100, incorporation of ^{13}C valine into albumin (FSR) was approximately four times greater in patients than controls (data not shown). Despite a reduced albumin pool, the ASR of albumin was significantly ($P < 0.0001$) increased from a mean of 72 ± 4 mg/kg/day in control subjects to 149 ± 20 mg/kg/day in the nephrotic patients (Table 3).

There was no relationship between the ASR of albumin and that of apo B100 in VLDL ($r^2 = 0.36$; $P = 0.13$) in the patient group. If we excluded the patient (No. 8) with a mild proteinuria (2.9 g/day) and a similar ASR of albumin to what was observed in the control group, the relationship becomes worse ($r^2 = 0.16$; $P = 0.38$). There was also no relationship between plasma albumin

Table 3. Kinetics of apo B100 and albumin

	Apo B100 from VLDL			Apo B100 from LDL			Albumin	
	FCR *%/hr*	Pool size *mg*	ASR *mg/kg/day*	FCR *%/hr*	Pool size *mg*	ASR *mg/kg/day*	Pool size *g*	ASR *mg/kg/day*
Patient no.								
1	5.9	1327	23	1.3	3659	14	69	158
2	9.5	1852	49	5.8	3172	50	87	179
3	12.3	822	33	1.3	5572	23	81	241
4	5.5	648	9	—	2395	—	75	126
5	28.2	490	39	1.1	3320	11	75	111
6	19.9	967	48	1.2	6078	18	94	194
7	47.2	170	27	3.2	4920	53	58	107
8	47.0	32	5	3.3	2397	28	79	71
Mean ± SEM	21.9	789[a]	29[a]	2.4	3939[b]	28[c]	77[d]	149[b]
	6.1	212	6	0.7	500	7	4	20
Controls								
9	18.8	138	8	1.1	1785	6	127	53
10	32.9	250	28	1.1	2070	8	118	70
11	22.7	143	9	1.0	2296	6	124	88
12	28.1	208	14	1.3	1966	6	125	63
13	37.7	170	23	1.9	1378	10	101	81
14	31.4	83	9	0.7	1343	3	109	77
15	13.8	38	2	1.0	961	4	93	71
Mean ± SEM	26.5	147	13	1.1	1686	6	114	72
	3.2	29	4	0.1	179	1	5	4

The parameters for VLDL and LDL apo B100 were estimated using a multicompartmental model as shown in Figure 1. The FCR of apo B100 LDL could not be established in patient no. 4. Abbreviations are in the **Appendix**.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, [d] $P < 0.0001$ significantly different from the control group

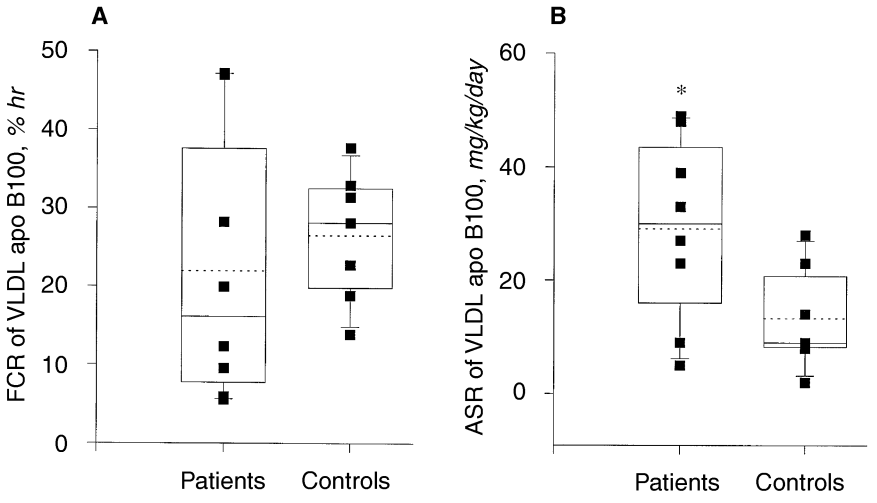

Fig. 2. Box plots showing the fractional catabolic rate (*A*; FCR, %/hr) and the absolute synthesis rate (*B*; ASR, mg/kg/day) of very low density lipoprotein (VLDL) apolipoprotein (apo) B100 in both patients and controls. Shown are the mean (black line), median (dotted line) and range (vertical bars). *$P < 0.05$.

concentration and the ASR of VLDL apo B100 ($r^2 = 0.20$; $P = 0.26$).

DISCUSSION

The present study showed that while some patients had increased secretion of VLDL apo B 100, hypertriglyceridemia in the nephrotic patients was mainly a result of a defect in VLDL apo B100 catabolism. The increase in absolute synthesis rate was incidental to increased plasma levels of VLDL, VLDL apo B100 or to increased levels of triglycerides. Synthesis of VLDL apo B100 was not significantly related to albumin synthesis or plasma albumin concentration. The LDL apo B100 pool was increased in all patients, and in contrast to the VLDL apo B100 pool, the FCR was not decreased. The ASR of LDL apo B100 was significantly increased in all patients. Indeed, the fractional turnover rate for LDL apo B100 was actually increased in the majority of our patients.

A defect in VLDL catabolism was also suggested in two small studies both performed in four nephrotic patients [3, 5]. Also, the observation by Warwick et al [2] suggested that a catabolic defect of VLDL apo B100 plays an important role in nephrotic dyslipidemia. The current study validates these findings and correlates impaired catabolism directly to hypertriglyceridemia in patients with a nephrotic syndrome. Physical and/or chemical changes of VLDL particles [30] as well as a decrease in LPL activity [31, 32] have been suggested as causes for impaired catabolism of VLDL. However, Davies et al showed a normal clearance of VLDL in hereditary analbumenic rats, which have a decreased LPL activity,

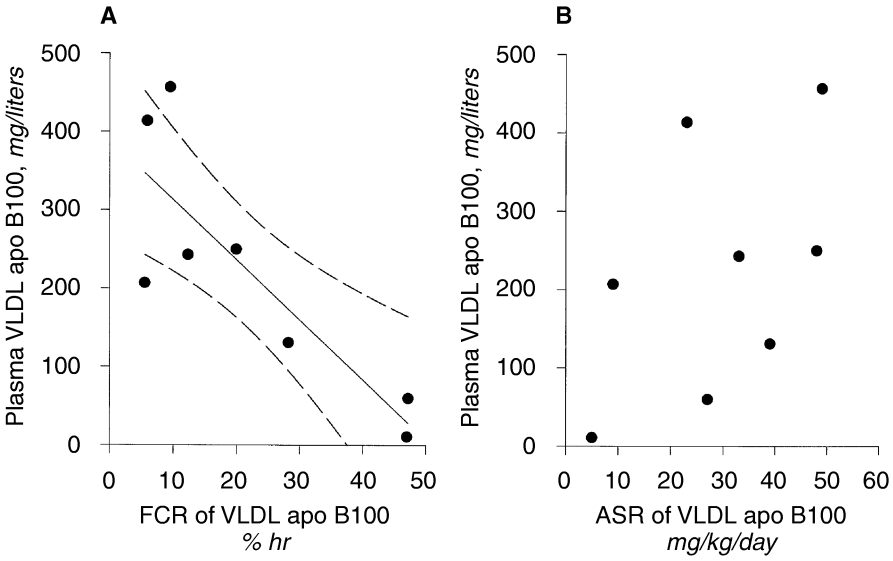

Fig. 3. Relationship between plasma very low density lipoprotein (VLDL) apolipoprotein (apo) B100 (mg/liter) and the fractional catabolic rate (FCR) of apo B100 (%/hr) in nephrotic patients (*A*; $r^2 = 0.708$, $P = 0.0088$). The 95% confidence limits of the whole group are shown on either side of the regression line ($N = 8$). Relationship between plasma VLDL apo B100 (mg/liter) and the absolute synthesis rate (ASR) of VLDL apo B100 (mg/kg/day) for nephrotic patients (*B*; $r^2 = 0.25$, $P = 0.21$; $N = 8$).

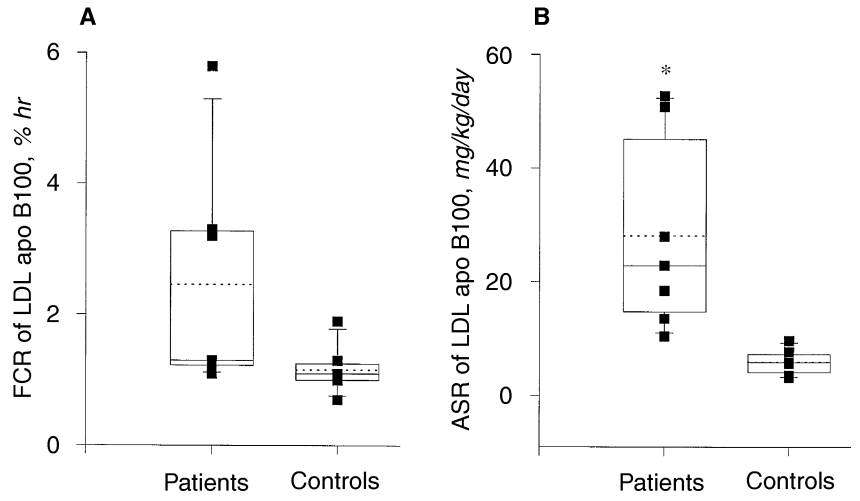

Fig. 4. Box plots showing the fractional catabolic rate (FCR %/hr; *A*) and the absolute synthesis rate (ASR) of low density lipoprotein (LDL) apolipoprotein (apo) B100 (mg/kg/day; *B*) in both patients and controls. Shown are the mean (black line), median (dotted line) and range (vertical bars) (*$P = 0.001$).

while the clearance of VLDL was significantly reduced when the nephrotic syndrome is imposed [33]. These observations suggest that a decrease in LPL activity may be less important in the nephrotic syndrome. As the IDL pool (the formation of which is also dependent upon LPL activity) is increased several times in this study as well as in the study of Aguilar Salinas et al [5], it is tempting to speculate that there is a decrease in the direct clearance of VLDL apo B100 in the nephrotic patients. Recently, a VLDL receptor was described that specifically bound apo E-containing lipoproteins such as VLDL and IDL, and which was expressed in peripheral tissue including heart and skeletal muscle, adipose tissue, kidney and brain but not in liver [34]. A decrease in this specific receptor may result in a reduced-mediated uptake of plasma VLDL and hence a reduced clearance of these lipoproteins from the plasma. This hypothesis is further supported by a recent study of Liang and Vaziri, who showed that the VLDL receptor was reduced in heart and skeletal muscle in puromycin induced nephrotic rats and that receptor protein was inversely related to plasma VLDL and triglyceride concentrations [35].

All of the patients in our study had an increased cholesterol concentration in all lipoprotein fractions containing apo B100. The increased LDL apo B100 concentration in these patients could not be correlated statistically to either the FCR of LDL apo B100 nor the ASR of LDL apo B100. However, in all patients the ASR of LDL apo B100 was significantly higher compared to the control group. Furthermore, the FCR of LDL apo B100 tended to be higher in patients compared to controls. One could postulate that this increased apo B synthesis reflects apo B100 input from the VLDL delipidation pathway. However, synthesis of LDL apo B100 was larger than synthesis of in VLDL apo B100 in some patients (No. 7 and 8), suggesting a direct synthesis of LDL bypassing VLDL-delipidation. If so, this shunt pathway may also exist in other patients, as the LDL apo B100 synthesis that is measured is derived both from LDL secreted through VLDL as well as possible alternative pathways. It is not possible to establish what fraction of the LDL apo B100 pool is derived from VLDL and what fraction of that pool is a consequence of the proposed alternate pathway of synthesis, since total LDL apo B100 synthesis

rate is a sum of the two pathways. The existence of direct LDL secretion and normal LDL catabolism is also suggested from the data from Vega and Grundy [3] and Aguilar Salinas et al [5]. By contrast, Warwick et al [2, 4] in two different studies showed a decreased catabolic rate of LDL as the main mechanism for the increased LDL pool. Notably, the latter study used exogenously labeled lipoproteins that may be subject to alternative catabolism.

Our present data show no clear relationship between the ASR of VLDL apo B100 and the ASR of albumin in nephrotic patients ($r^2 = 0.36$; $P = 0.13$). Although more studies need be done in order to exclude a definite relationship, the lack of correlation is strikingly different from that between fibrinogen synthesis and albumin synthesis reported previously by us [18]. In addition, regulation of hepatic apo B100 secretion does not appear to be modulated transcriptionally, but rather translationally or post-translationally [36], while albumin is transcriptionally regulated [37]. We also found no relationship between apo B100 secretion in LDL and albumin synthesis in the nephrotic patient ($r^2 = 0.02$; $P = 0.78$). Since the LDL apo B100 pool is at least in part derived from VLDL, it is possible that direct synthesis and secretion of LDL may parallel that of albumin, but we have not tested this hypothesis directly since we cannot distinguish between sources of the LDL pool secreted through VLDL and sources secreted by alternate pathway(s). Since it was suggested that a low COP [10, 20], albumin or proteinuria [10, 38, 39] may play a role in apo B100 metabolism, we tested these relationships, but none of them achieved statistical significance.

The disturbances in lipoprotein profile in the nephrotic syndrome may not only be relevant to the increased risk of coronary atherosclerosis in these patients, but may also play a role in the progression of renal disease [40–44]. Our data indicate that the therapies aimed at induction of the VLDL receptor or suppression of secretion of VLDL (such as microsomal triglyceride transfer protein inhibitors or the new HMG-CoA reductase inhibitor, atorvastatine) could be more successful than current standard therapy to achieve full restoration of dyslipidemia in these patients.

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APPENDIX

Abbreviations used in this article are: apo, apolipoprotein; ASR, absolute synthesis rate; chol, cholesterol; COP, colloid oncotic pressure; FCR, fractional catabolic rate; FSR, fractional rate of synthesis; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein, NS, nephrotic syndrome; TG, triglyceride; VLDL, very low density lipoprotein.

REFERENCES

1. Kaysen GA: Hyperlipidemia of the nephrotic syndrome. *Kidney Int* 31(Suppl):S8–S15, 1991
2. Warwick GL, Packard CJ, Demant T, Bedford DK, Boulton-Jones JM, Shepherd J: Metabolism of apolipoprotein B-containing lipoproteins in subjects with nephrotic-range proteinuria. *Kidney Int* 40:129–138, 1991
3. Vega GL, Grundy SM: Lovastatin therapy in nephrotic hyperlipidemia: Effects on lipoprotein metabolism. *Kidney Int* 33:1160–1168, 1988
4. Warwick GL, Caslake MJ, Boulton Jones JM, Dagen M, Packard CJ, Shepherd J: Low-density lipoprotein metabolism in the nephrotic syndrome. *Metabolism* 39:187–192, 1990
5. Aguilar Salinas CA, Barrett PH, Kelber J, Delmez J, Schonfeld G: Physiologic mechanisms of action of lovastatin in nephrotic syndrome. *J Lipid Res* 36:188–199, 1995
6. Keane WF, Mulcahy WS, Kasiske BL, Kim Y, O'Donnell MP: Hyperlipidemia and progressive renal disease. *Kidney Int* 39(Suppl): S41–S48, 1991
7. Kasiske BL, O'Donnell MP, Cowardin W, Keane WF: Lipids and the kidney. *Hypertension* 15:443–450, 1990
8. Keane WF, O'Donnell MP, Kasiske BL, Schmitz PG: Lipids and the progression of renal disease. *J Am Soc Nephrol* 1(Suppl):S69–S74, 1990
9. Ohta T, Matsuda I: Lipid and apolipoprotein levels in patients with nephrotic syndrome. *Clin Chim Acta* 117:133–143, 1981
10. Appel GB, Blum CB, Chien S, Kunis CL, Appel AS: The hyperlipidemia of the nepfrotic syndrome. Relation to plasma albumin concentration, oncotic pressure, and viscosity. *N Engl J Med* 312:1544–1548, 1985
11. Parhofer KG, Barrett PHR, Bier DM, Schonfeld G: Determination of kinetic parameters of apolipoprotein B metabolism using amino acids labeled with stable isotopes. *J Lipid Res* 32:1311–1323, 1991
12. Cohn JS, Wagner DA, Cohn SD, Millar JS, Schaefer EJ: Measurement of very low density and low density lipoprotein apolipoprotein (Apo) B-100 and high density lipoprotein Apo A-I production in human subjects using deuterated leucine. Effect of fasting and feeding. *J Clin Invest* 85:804–811, 1990
13. Sun X, Kaysen GA: Albumin and transferrin synthesis are increased in H4 cells by serum from analbuminemic or nephrotic rats. *Kidney Int* 45:1381–1387, 1994
14. Lewandowski AE, Liao WSL, Stinson-Fisher CA, Kent JD, Jefferson LS: Effects of experimentally induced nephrosis on protein synthesis in rat liver. *Am J Physiol* 254:C634–C642, 1988
15. Kaysen GA: Albumin metabolism in the nephrotic syndrome: the effect of dietary protein intake. *Am J Kidney Dis* 12:461–480, 1988
16. Ballmer PE, Weber BK, Roy Chaudhurry P, McNurlan MA, Watson H, Power DA, Garlick PJ: Elevation of albumin synthesis rates in nephrotic patients measured with leucine. *Kidney Int* 41:132–138, 1992
17. Kaysen GA, Gambertoglio J, Jimenez I, Jones H, Hutchison FN: Effect of dietary protein intake on albumin homeostasis in nephrotic patients. *Kidney Int* 29:572–577, 1986
18. de Sain-van der Velden MGM, Kaysen GA, de Meer K, Stellaard F, Voorbij HAM, Reijngoud DJ, Rabelink TJ, Koomans HA: Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients. Measurements with stable isotopes. *Kidney Int* (in press)
19. Marsh JB, Sparks CE: Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. *J Clin Invest* 64:1229–1237, 1979
20. Davis RA, Engelhorn SC, Weinstein DB, Steinberg D: Very low density lipoprotein secretion by cultured rat hepatocytes. Inhibition by albumin and other macromolecules. *J Biol Chem* 255:2039–2045, 1980
21. Freinkel N, Schreiner GE, Athens JW: Simultaneous distribution of T-1824 and I^{131}-labelled human serum albumin in man. *J Clin Invest* 32:138–148, 1953
22. Pekelharing HLM, Kleinveld HA, Duif PFCCM, Bouma BN, Van Rijn HJM: Effect of lipoprotein(a) and LDL on plasminogen binding to extracellular matrix and on matrix-dependent plasminogen activation by tissue plasminogen activator. *Thromb Haemost* 75:497–502, 1996
23. Egusa G, Brady DW, Grundy SM, Howard BV: Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of

very low density lipoprotein and low density lipoprotein apolipoprotein B. *J Lipid Res* 24:1261–1267, 1983
24. HILL RL: Hydrolysis of proteins. *Advantages of Prot Chem* 20:37–107, 1965
25. REDGRAVE TG, ROBERTS DCK, WEST CE: Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal Biochem* 65:42–49, 1975
26. VÄISÄNEN S, GÄVERT J, JULKUNEN A, VOUTILAINEN E, PENTTILÄ I: Contents of apolipoprotein A-I, A-II and B of the human serum fractions for high-density and low-density lipoproteins prepared by common precipitation methods. *Scand J Clin Lab Invest* 52:853–862, 1992
27. HUSEK P: Rapid derivatization and gas chromatographic determination of amino acids. *J Chromatogr* 552:289–299, 1991
28. *SAAM II User Guide*. Seattle, University of Washington
29. FOSTER DM, BARRETT PHR, TOFFOLO G, BELTZ WF, COBELLI C: Estimating the fractional synthetic rate of plasma apolipoproteins and lipids from stable isotope data. *J Lipid Res* 34:2193–2205, 1993
30. FURUKAWA S, HIRANO T, MAMO JCL, NAGANO S, TAKAHASHI T: Catabolic defect of triglyceride is associated with abnormal very-low-density lipoprotein in experimental nephrosis. *Metabolism* 39:101–107, 1990
31. YAMADA M, MATSUDA I: Lipoprotein lipase in clinical and experimental nephrosis. *Clin Chim Acta* 30:787–794, 1970
32. CHAN MK, PERSAUD JW, VARGHESE Z, MOORHEAD JF: Post-heparin hepatic and lipoprotein lipase activities in nephrotic syndrome. *Aust NZ J Med* 14:841–847, 1984
33. DAVIES RW, STAPRANS I, HUTCHISON FN, KAYSEN GA: Proteinuria, not altered albumin metabolism, affects hyperlipidemia in the nephrotic rat. *J Clin Invest* 86:600–605, 1990
34. TAKAHASHI S, KAWARABAYASI Y, NAKAI T, SAKAI J, YAMAMOTO T: Rabbit very low density lipoprotein receptor: A low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc Natl Acad Sci USA* 89:9252–9256, 1992
35. LIANG KH, VAZIRI ND: Acquired VLDL receptor deficiency in experimental nephrosis. *Kidney Int* 51:1761–1765, 1997
36. PULLINGER CR, NORTH JD, TENG BB, RIFICI VA, RONHILD DE BRITO AE, SCOTT J: The apolipoprotein B gene is constitutively expressed in HepG2 cells: Regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J Lipid Res* 30:1065–1077, 1989
37. KAYSEN GA, JONES H JR, MARTIN V, HUTCHISON FN: A low-protein diet restricts albumin synthesis in nephrotic rats. *J Clin Invest* 83:1623–1629, 1989
38. CIANFLONE K, VU H, ZHANG Z, SNIDERMAN AD: Effects of albumin on lipid synthesis, apo B-100 secretion, and LDL catabolism in HepG2 cells. *Atherosclerosis* 107:125–135, 1994
39. APPEL G: Lipid abnormalities in renal disease. *Kidney Int* 39:169–183, 1991
40. BERGESIO F, MONZANI G, CIUTI R, SERRUTO A, BENUCCI A, FRIZZI V, SALVADORI M: Lipids and apolipoproteins change during the progression of chronic renal failure. *Clin Nephrol* 38:264–270, 1992
41. DIAMOND JR, KARNOVSKY MJ: Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int* 33:917–924, 1988
42. SAMUELSSON O, AURELL M, KNIGHT-GIBSON C, ALAUPOVIC P, ATTMAN P: Apolipoprotein-B-containing lipoproteins and the progression of renal insufficiency. *Nephron* 63:279–285, 1993
43. KEANE WF: Lipids and the kidney. (Perspectives in Clinical Nephrology) *Kidney Int* 46:910–920, 1994
44. SATO H, SUZUKI S, KOBAYASHI H, OGINO S, INOMATA A, ARAKAWA M: Immunohistological localization of apolipoproteins in the glomeruli in renal disease: Specifically apoB and apoE. *Clin Nephrol* 36:127–133, 1991